

LARVICIDAL EFFECT OF SOME PLANT EXTRACTS AGAINST TOMATO LEAF MINER (*TUTA ABSOLUTA* MEYRICK; LEPIDOPTERA: GELEHIIDAE)

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Tuta absoluta Meyrick (Lepidoptera: Gelehiidae) is a significant tomato pest, and chemical pesticides are used extensively for its control. Because of the adverse effects of chemical pesticides, researchers have been paying attention more to biopesticides. In this study, the larvicidal effects of the extracts of *Tanacetum vulgare* (Asteracea), *Aleo vera* (L.) and (Liliaceae) *Tagetes patula* L. (Asteracea) was determined and trials were conducted by using leaf dipping and pot methods. The leaf dipping method was conducted in a climate chamber, whereas the potting experiment was performed in the greenhouse. In all of the experiments, second-stage larvae were used to determine the larvicidal effects. According to the results obtained, the highest larvicidal effect and mortality rate was observed at the highest concentration of *T. vulgare* extract (86%, effect: 86.55%). The lowest mortality rate and the larvicidal effect was observed at the same concentration of *T. patula* extract (mortality: 79%, effect: 78.55%). In the pot experiment, the highest yield was seen in the Neem Azal T/S, followed by *T. vulgare*, *A. vera* and *T. patula* extracts. On the first day counts, the lowest insecticidal effect was obtained with the lowest concentration of *T. patula* extract. The extract of *A. vera*, *T. vulgare* and *T. patula* was effective in experiments carried out in pot and laboratory conditions on *T. absoluta*. It is suggested that more research be done on using these extracts as a biopesticide to control *T. absoluta*.

Keywords: Tomato leaf miner, plant extracts, larvicidal effect.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important crop that has a crucial role in human food consumption. It contains many vitamins such as B-12, A, D, and E beneficial for human health. Tomato is produced in almost all regions of Turkey and is one of the primary export products of Turkey. Tomato has many harmful pests causing significant losses in tomato production in Turkey. In 2009, *T. absoluta* was observed in Turkey and thereafter, it is considered as preeminent tomato pest in all tomato grown regions of Turkey. Tomato exports were severely affected due to high yield losses caused by *T. absoluta*. Despite applying all control methods against *T. absoluta*, it still poses a serious threat to tomato farmers and country export. Mediterranean climate provides suitable conditions for *T. absoluta*, generating 10-12 offspring per year. The life cycle of *T. absoluta* lasts around 30-35 days, with females leaving around 260 eggs during their lifespan (Franca, 1993). It attacks potatoes, eggplants, pepinos, artichokes, beans and tobaccos (Pereyra *et al.*, 2006). The

larvae of *T. absoluta* feed on leaf mesophyll tissues and penetrate stems and fruits in every phonological period. Moreover, fungi and bacteria can enter the galleries opened in fruit and as a result, the product becomes unusable. *T. absoluta* decreases the quality of tomatoes grown outdoors and in greenhouses and the product losses become immense in heavily infected areas (Eppo, 2005). After *T. absoluta* was observed in Turkey, the Republic of the Ministry of Agriculture and Forestry recommended many chemical insecticides to control it. Although there are different methods to control *T. absoluta*, such as biological, biotechnical, and environmentally friendly insecticides, tomato farmers prefer to use broad-spectrum insecticides for *T. absoluta* control. The usage of chemical pesticides against *T. absoluta* caused the development of resistance in a short time. Moreover, the widespread use of insecticides caused environmental pollution and the remaining product residue. Due to the adverse effects of chemical pesticides, scientists have shifted the focus to biopesticides (Dogan *et al.*, 2017). Natural materials like plants (Ahmad *et al.* 2019), animals, bacteria



and some minerals can be used as a source of biopesticides. Baking soda and canola oil have pesticidal actions and can be considered as examples of biopesticides. (Thangavel and Sridevi, 2015). Most of the work has been conducted on plants. The substances extracted from some plants have been used for many years to control insects.

Plant compounds can have insecticide, repellent, antifeeding, oviposition deterrence and growth retardant effects on insects. Botanical insecticides can selectively target insects while not affecting their natural enemies, thus providing a safe environment and residue-free food (Emsen *et al.*, 2016; Ahmad *et al.*, 2019). *T. vulgare* is a traditional medicinal plant used to cure fevers, headaches, migraine, insect bites, toothaches, stomachaches, rheumatoid arthritis, infertility, menstruation problems, and childbirth labour (Pareek *et al.*, 2011). It is a perennial plant that was used traditionally to cure various ailments. Parts of *T. parthenium* contains essential oils, bitter glycosides, lactones, flavonoids and tannins. Generally, the leaves of the plant are hung in some places in the house to repel flies. The dried thin branches are placed under the carpets and spread to suitable places in the kitchen and cellar to abduct ants and mice (Drury, 1992). The variability in the chemical composition of essential oils of *T. vulgare* has been documented (Cote *et al.*, 2017).

A. vera is the valuable medicinal plants which can tolerate dryness and high temperatures. Many compounds, such as amino acids, choline, enzymes, metabolic and phenolic compounds, minerals and sugars, can be obtained from *A. vera* (Raksha *et al.*, 2014). *A. vera* extract also exhibited high acaricidal and repellent impact on *Tetranychus cinnabarinus* Boisduval (Acarina: Tetranychidae) (Zhang *et al.*, 2013). *T. patula* L. is a medicinal plant used in several cultures as florets or infusions from dried leaves (Soule, 1993). Secondary metabolites in flowers, seeds, and roots of *Tagetes* species involve β -karyophyllene, methyl eugenol, limonene, anetol and alilanol. These metabolites have toxic effects on bacteria, fungi, viruses, mites, insects, and nematodes. The extracts of *T. patula* and *Tagetes minuta* can be used as insecticides, fungicides and nematocides (Welty and Prestbye, 1993; Philogene *et al.*, 1985; Miller and Ahrens, 1969). Camarillo *et al.* (2007) reported that the compounds of *Tagetes* are essential oil, alcohols, aldehydes, carotenoids, ethers, esters, flavonoids, ketones, and thiophenes and belong to specific groups of hydrocarbons. It was determined that ethanolic leaf extracts of *T. patula* have a strong insecticidal effect on all stages of *Aedes aegypti* L. (Vidal, 2009). Ramagnoli *et al.* (2005) investigated the essential oil of *T. patula* and documented the antifungal activity against *Botrytis cinerea* and *Penicillium digitatum*. Moreover, Mares *et al.* (2004) indicated that the extract of *T. patula* showed growth inhibition in *B. cinerea*, *Fusarium moniliforme* and *Pythium ultimum*. This research work aims to evaluate the insecticidal effects of some plant extracts on *T. absoluta*.

MATERIALS AND METHODS

Tomato leaf miner culture: *T. absoluta* larvae were obtained from tomato greenhouses in Ankara. The *T. absoluta* larvae were kept along with a tomato plant in the cage with size of 50×50×30 cm. The adults (newly emerged) were relocated into the different cages with tomato plants. Tomato plants used in this study were grown under greenhouse conditions. The joker variety of tomato was used for stock culture (*T. absoluta*) and for experiments,

Plant extracts: *T. vulgare* were obtained in 2018 in the province of Ankara. Plants and *T. vulgare* were both obtained while growing actively at the flowering stage. All the aboveground parts of the plants were utilized to acquire the extract. *A. vera* was grown in pots at room temperature. *T. patula* was collected from parks in Ankara. Neem Azal T/S was purchased from the company and used as a positive control.

Table 1. Plants used to evaluate the insecticidal effect on *Tuta absoluta*

| Scientific name | Family name | Tissue used |
|-----------------------------|-------------|-----------------------|
| <i>Tanacetum vulgare</i> L. | Asteraceae | Flowers, leaves, buds |
| <i>Aloe vera</i> (L.) | Liliaceae | Leaves |
| <i>Tagetes patula</i> L. | Asteraceae | Flowers, leaves |

Preparing the extracts: Plant material collected were cleaned and dried in shadow. After the plant was dried, it was cut into small pieces in the mill. 400 mL of ethanol (80%) and 200 g plant material (powdered) were added to prepare the extract. After 72 hours, the mixture was placed into Soxhlet for 5-6 hours. Then it was filtered with Whatman No.1 filter paper a Bucher funnel. A rotary evaporator (50-60 °C) was used to concentrate the extracts by evaporation under low pressure. Prepared plant extracts were kept at 4 °C in glass vials for later usage as stocks.

Larvacidal Effect of Plant Extracts on larva of *T. absoluta*

Leaf-dipping method: Discs of 3 cm diameter were punched out from fresh untreated tomato leaf and dipped into the test solutions prepared from extracts (*T. vulgare*, *T. patula*, *A. vera*; 1, 2.5, 5 and 10% and Neem Azal T/S 0.5%) for one minute. All solutions were prepared with distilled water (dH₂O) and 0.01% Triton X-100 was added. The control disc was dipped in 0.01% Triton X-100 solution. After 30 minutes to dry, control and treated leaf discs were transferred to new Petri dishes provided with moistened filter paper. After that, 5-second stage larvae of *T. absoluta* were placed onto the discs. Ten replicates were conducted during the experiment, including the control. Petri dishes were kept in a climate chamber. Counts after 1, 3 and 7 days were conducted as alive or dead and recorded.

Pot experiment: Tomato seedlings were grown in the climate chamber by planted in pots and placed in the greenhouse, with controlled conditions (20±5 °C, 60-65% humidity) for their

development. All the pots were placed in a large cage after tomato plants developed. Then, adults of the same age (1-3 days) were left in the cage and kept together for 24 hours for mating (50 females + 50 males). The pots were removed from the cage after adults were allowed to lay eggs for forty-eight hours. The eggs on the plants in the pot were counted with a magnifying glass and recorded. At least 20 to 30 eggs were left in each plant. The pots were placed in the greenhouse for eggs to hatch and develop to larvae. Due to the biology of tomato leaf, miner second-stage larvae were counted in the plants. The count of living larvae on each plant was recorded. The prepared concentrations of the extracts were sprayed to the plants using a small hand sprayer. The pots were placed in the greenhouse according to the trial pattern of random parcels to determine the extract effect on larvae. Counts were conducted after 3, 7 and 14 days and larvae were recorded as dead or alive. The trial was set up with four replications. Plants in control pots were sprayed with water. The amount of water consumed for each plant was determined by calibration.

Statistical evaluation: Abbott (1925) analysis was used to calculate The variance analysis, the mean values were compared by Duncan's test ($P = 0.05$) using the obtained results by using SPSS 20.6 statistical software. The mortality rate was calculated as mortality = the number of dead larvae after treatment/number of larvae before treatment the.100).

RESULTS

Leaf-dipping method: The values in table 2 indicate that the highest mortality rate was observed in Neem Azal T/S treatment. The highest mortality among the three extracts was from *T. vulgare* extract treatment. The lowest mortality rate was obtained at the lowest concentration of *A. vera* extract. The highest effect was noticed with Neem Azal T/S. The highest effect was recorded among the extracts at the highest concentration (10%) of *T. vulgare* extract. This value was

followed by an extract of *A. vera*. The lowest effect was observed in the *T. patula* extract. According to statistical analysis, the highest concentrations of all of the extracts were in the same group. Other extracts concentrations formed variable groups (Table 2) ($F = 22.74$; $P < 0.05$).

Table 2. The mortality rate of *T. absoluta* on extracts of three different plants (mean \pm SE).

| Treatments | Concetration (%) | Mortality (%) | Effect (%) |
|--------------------------------------|------------------|---------------|--------------------|
| <i>Aloe vera L.</i> | 1 | 23 | 21.22 \pm 3.65d* |
| | 2.5 | 39 | 38.11 \pm 3.71c |
| | 5 | 57 | 55.66 \pm 1.68b |
| | 10 | 83 | 82.55 \pm 2.45a |
| <i>Tegetes patula L.</i> | 1 | 28 | 26.44 \pm 2.35d |
| | 2.5 | 48 | 47.00 \pm 3.12c |
| | 5 | 61 | 60.22 \pm 3.11b |
| | 10 | 79 | 78.55 \pm 2.09a |
| <i>Tanacetum vulgare L.</i> | 1 | 52 | 50.66 \pm 3.78b |
| | 2.5 | 79 | 78.55 \pm 2.56a |
| | 5 | 81 | 81.77 \pm 2.24a |
| | 10 | 87 | 86.55 \pm 2.34a |
| Neem Azal T/S (% 10 azadirachtin) | 3 | 98 | 97.88 \pm 1.98a |
| | Control | 2 | |

*Means within rows followed by the same uppercase letter are not significantly different (Duncuns's multiple range test).

Pot experiments: All the insecticidal effect data related to plant extracts are shown in Table 3. The maximum effect was observed with the Neem Azal T/S used as the standard commercial product, followed by the extracts of *T. vulgare*, *A. vera* and *T. patula*. On the first day counts, the lowest insecticidal effect was observed with the lowest concentration of *T. patula* extract. The highest insecticidal effect was that of the Neem Azal T/S of a commercial product. Similar results were obtained on other count days. In statistical analysis, the highest concentrations of all extracts were observed on 7th day from the same group on ($F = 23.124$; $P < 0.05$).

Table 3. The larvacidal effects of extracts three different plants on *T. absoluta* (mean \pm SE).

| Treatments | Concentrations (%) | Count Times | | |
|-----------------------------|--------------------|------------------------|-----------------------|----------------------|
| | | 1st day | 3rd day Effect (%) | 7th day |
| <i>Aleo vera L.</i> | 5 | 33.23 \pm 2.44 c*C** | 43.77 \pm 2.54 b C | 49.89 \pm 1.78 a C |
| | 10 | 47.29 \pm 2.89 b C | 56.23 \pm 3.24 b B | 62.26 \pm 1.39 aB |
| | 12.5 | 58.41 \pm 1.66 a A | 76.18 \pm 2.13 a A | 82.31 \pm 1.63 a A |
| <i>Tagetes patula L.</i> | 5 | 24.44 \pm 2.63 c C | 37.11 \pm 1.73 c C | 42.19 \pm 0.92 c C |
| | 10 | 33.71 \pm 1.65 c C | 45.92 \pm 2.78 b B | 51.34 \pm 1.12 b C |
| | 12.5 | 59.46 \pm 1.55 a A | 65.32 \pm 1.65 a A | 79.26 \pm 1.59 a A |
| <i>Tanacetum vulgare L.</i> | 5 | 35.76 \pm 2.67 c C | 44.47 \pm 2.56 b C | 52.49 \pm 1.43 b C |
| | 10 | 38.34 \pm 2.33 c C | 62.89 \pm 2.64 b B | 61.33 \pm 2.53 a A |
| | 12.5 | 61.12 \pm 2.77 b A | 73.40 \pm 1.85 a A | 84.32 \pm 0.71 a A |
| Neem Azal T/S | 5 | 72.43 \pm 2.73 C | 83.47 \pm 1.32 B | 92.31 \pm 0.81 A |

*Means within rows followed by the same uppercase letter are not significantly different (Duncuns's multiple range test), ** Means within a column followed by the identical lowercase are not significantly different (Duncuns's multiple range test).

DISCUSSIONS

A. vera is a potential plant exhibiting activities like preventative bio-pesticide and growth enhancer (Omotaso, 2008). Moreover, secondary metabolite screening was performed on *A. vera* extracts where alkaloids, saponins, tannins, flavonoids, and glycosides were found to be present (Pedro *et al.*, 2014; Ramesh *et al.*, 2013). Alkaloids were used as a stomach poison. They also have growth inhibition effects against insects, particularly of the three insect hormones, i.e. the ecdysis hormone, the brain hormone, and the juvenile hormone, which can cause metamorphosis failure (Lumowa and Nova, 2014). The leaf extract and gel of *A. vera* has antifungal, antibacterial, anticancer, antioxidant, cry protective, immune-modulatory and insecticidal activities (Patel *et al.*, 2012, Morsy *et al.*, 2000). Shivakumar *et al.* (2013) reported that secondary metabolites like alkaloids, flavonoids, tannins and saponins of *A. vera* leaves exhibit larvicidal effect. Results revealed the larvicidal effects of *A. vera* extract due to the presence of toxic substances to mosquito larvae with the LC50 value of 80.5 ppm at 1440 min (Romasepfani *et al.*, 2018). Sarwar (2013) noted that *A. vera* extract has the most significant effect on *Musca domestica* L. larvae. Moreover, the *A. vera* extract showed a repellent impact on *Sitophilus oryzae* L. Whereas *A. vera* extract exhibited the maximum mortality rate against *Sitotrage cerealella* L. (Lep.:Gelechiidae). McCloskey *et al.* (1993) revealed that *A. vera* carbon tetrachloride extract exhibited larvicidal effect against *Culex quinquefasciatus* with LC50 of 15.31 ppm after 24 h. Bekele and Petros (2017) reported that *Aloe pirottae* and *Brassica nigra* extracts have reliable efficacy in mosquito repellency on *Anopheles arabiensis* Patton (Diptera: Culicidae). Erdogan (2019) revealed that the extracts of *A. vera* have a deterrence effect of 94.68% on *T. absoluta*. The crude extract of *A. vera* was more active in terms of larvicidal potential against *Musca Domestica* (Jesikha, 2012).

The effects of *T. vulgare* extract on various insects have been documented in different studies. Goldstain and Goldstein (1992) stated that *T. vulgare* aqueous extract extends the duration of the pupa of *Pieris rapae* (L.) and *Plutella xylostella* (L.), and adults lay three times less eggs than the control. Erdogan (2019) revealed that the *T. vulgare* extract caused a decrease in the egg count of *T. absoluta*. Chiossan *et al.* (2001) suggested that *T. vulgare* extract showed the maximum acaricidal activity on *Tetranychus urticae*. In the same study, after the chemical analysis of *T. vulgare* extract was carried out, it was found that it contains a substance called beta-thujone. This substance is the main component of its oil (87.6%). It was demonstrated that the mite has a high mortality effect in this oil. Similarly, it was revealed that *T. vulgare* contains casticin, eupatilin, casticin, acacetin, quercitrin and isoquercitrin (Ivanescue *et al.*, 2018). Moreover, the studies revealed that the contents of *T. vulgare*

include bornyl acetate, 1,8-cineole, γ -terpinene, p-cymene, and camphor. It is noted that each of these substances exerts a strong repellent effect on the potato beetle (Scheerer, 1984). The study conducted by Magierowicz *et al.* (2020) reported that larvae of *Leptinotarsa decemlineata* fed with treated extract *T. vulgare* food lay less eggs than the control. In another study, it was determined that extract of *T. parthenium*, which is in the same family as *T. vulgare* showed the highest mortality (87%) at the highest concentrations (12%) on Potato tuber moth [*Phthorimae operculella* Zeller (Lep.: Gelechiidae)] (Erdogan *et al.*, 2018). Earlier, Erdogan *et al.* (2012) reported that the *T. parthenium* extract has a high mortality rate against *P. operculella*, the adult mites laid lower numbers of eggs than the untreated control and no ovicidal effect was detected. The extract of *T. vulgare* formulated had a strong mortality effect on larvae of *Choristoneura rosaceana* Harris (Lep.: Tortricidae) (Larocque *et al.*, 1999). Nilahyan *et al.* (2012) suggested that among the extracts, the highest rates of mortality were observed from *T. vulgaris* leaves (95%), and the LD90 of these extracts showed that the *T. vulgaris* ethanol extract was the most toxic (LD90 = 89383mg/l) on *T. absoluta*.

Essential oil of *T. patula* can be utilized as a residual pesticide against bedbugs (Politi *et al.*, 2017). *T. patula* extract is also known to contain thiophene derivatives (Bicchi *et al.*, 1992). The essential oil of *T. patula* was also investigated for the antifungal activity, including treating fungal infections in plants and the candidiasis treatment (Romagnoli *et al.*, 2005; Mares *et al.*, 2004). Rajasekaran *et al.* (2004) revealed that *T. patula* extract showed a larvicidal effect against mosquito larvae. The extract of *T. patula* caused significantly reduced survival of *Latrodectus hesperus* and *Bemisia tabaci* species on plants (Fabric, 2020). The extract of *T. patula* had a mortality rate of 65.5% on *Aedes fluviatilis* (Diptera: Culicidae) (Macedo, 1997). Another study carried out by Sánchez *et al.* (2012) revealed that three leaf extracts of *Tagetes erecta* L. (Asteracea) caused with hexane, acetone and ethanol have high larval and pupal mortality on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Phoofolo *et al.* (2013) reported that crude extracts of *T. minuta* caused significant fecundity reduction as the crude extract concentration increased. The same authors determined that the extract exhibited the significant fecundity reduction with elevated crude extracts concentration and had the most substantial effect on fecundity on *T. absoluta*. Ravikumar (2010) found that *T. erecta* was the most active after *T. patula*, and in the insecticidal bioassays, the best result occurred in different concentration of *T. erecta* flower oil to *Acyrtosiphon gossypii* and *S. frugiperda*. *T. minuta* root extract had a mortality effect on different aquatic macroinvertebrates (Kumar *et al.*, 2000). Kyo *et al.* (1990) indicated that the extract of *A. vera* of the nematicidal activity was due predominantly to 6-terthienyl.

Conclusion: In this study, the extract of *A. vera*, *T. vulgare* and *T. patula* were effective in experiments carried out in both pot and laboratory conditions on *T. absoluta*. It is suggested that more research be carried out on using these extracts as a biopesticide to control *T. absoluta*.

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